Supplementary Figure S3

A

H446

Direct irradiation

DNA replication

Relative expression level

CDKN1A
GADD45A
TIGAR
AEN
MCM2
MCM4
PCNA

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H82

DNA replication

Direct irradiation

Relative expression level

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B

H446

p-H2AX

DAPI + p-H2AX

Scr

sgDHX9

H1048

p-H2AX

DAPI + p-H2AX

Scr

sgDHX9

D

H82

Scr sgDHX9

H1048

Scr sgDHX9

DHX9

p-CHK1

p-CHK2

p-H2AX

clePARP

β-actin

C

DAPI + DNA/RNA hybrid

DNA/RNA hybrid

DAPI + DNA/RNA hybrid

Rnase H (-)

Scr

sgDHX9

Rnase H (+)

D

Scr sgDHX9

RNase H1

D

Scr sgDHX9

DHX9

p-CHK1

p-CHK2

p-H2AX

clePARP

V5 tag (RNase)

β-actin
Supplementary Figure S3 (continued)

F  siCtrl  siDHX9
GFP  GFP  WT  K417R
Flag
DHX9
β-actin

G
Relative cell number

siCtrl  siDHX9  siDHX9+WT  siDHX9+K417R

H
DNA/RNA hybrid

fluorescence intensity

siCtrl  siDHX9  siDHX9+WT  siDHX9+K417R

I
% of stalled forks

Scr  sgDHX9  RNase H1  +sgDHX9

J
Enrichment score

Senescence

NES = 1.04
p value = 0.38

Scr  sgDHX9

K
Relative expression level

JUN  CDKN1A  CDKN2A

Senescence

Scr  sgDHX9
Supplementary Figure S3.

A, qRT-PCR analysis of the direct irradiation response and replication-related genes comparing Scramble and sgDHX9 of H446 and H82 cells (n = 3). 36B4 gene was used as a reference. B, Immunofluorescence images of p-H2AX (red) staining of Scramble and sgDHX9 of H446 and H1048 cells. Scale bar = 50 μm. C, Immunofluorescence images of DNA/RNA hybrid (red) staining of Scramble and sgDHX9 H196 cells, treated w/wo RNase H. Scale bar = 25 μm. D, Immunoblot (IB) of the indicated proteins in Scramble and sgDHX9 of H82 and H1048 cells. E, Immunoblot (IB) of the indicated proteins in Scramble and sgDHX9 H82 cells, w/wo overexpression of RNase H1-V5. F, Immunoblot (IB) of the indicated proteins in H446 cells overexpressing 3xFlag-DHX9 (WT/K417R) or Flag-GFP, transfected with siCtrl or siDHX9-3'UTR. G, Relative cell number of H446 cells treated with the indicated siRNAs and expression vectors. Luminescence of CellTiter-Glo was detected on Day 4 after seeding (n = 3). H, Fluorescence intensity of DNA/RNA hybrid in H446 cells treated with the indicated siRNAs and expression vectors, was quantified (100 cells were counted per group). I, The percentage of stalled forks over the total number of different replication structures was measured (>150 labeled forks were counted per group, n = 3). J, GSEA analysis with C2 (curated) gene sets, based on RNA-seq results of sgDHX9 versus Scramble cells. K, qRT-PCR analysis of the senescence-related genes comparing Scramble and sgDHX9 H196 cells (n = 3). 36B4 gene was used as a reference.

Data represent mean ± SEM. ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by unpaired Student’s t test (A and K) and one-way ANOVA (G, H and I).